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Agronomic and Seed Traits of Soybean Lines with Low–Phytate Phosphorus

Sheilah E. Oltmans, Walter R. Fehr,* Grace A. Welke, Victor Raboy, and Kevin L. Peterson

ABSTRACT

About 75% of the total P in conventional soybean [*Glycine max* (L.) Merr.] seed is phytate P, which cannot be readily digested by nonruminant livestock, such as swine and poultry. The phytate P in soybean lines homozygous for the recessive alleles *pha1* and *pha2* is reduced to about 25% of the total P. The objective of this study was to determine the influence of low phytate (LP) on agronomic and seed traits of soybean. Three populations were developed by crossing three cultivars with normal phytate (NP) to the LP line CX1834-1-6. From each population, 10 LP and 10 NP lines were selected and grown in replicated tests at three Iowa environments during 2003. The mean total P of the LP and NP lines was not significantly different, but the mean phytate P, inorganic P, and other P were significantly different for the two types of lines in the three populations. The mean seedling emergence of the LP lines was 45% compared with 68% for the NP lines. The mean differences between the LP and NP lines for the other agronomic and seed traits were not significant in one or more of the populations. On the basis of these results, reduced seedling emergence will be a major factor to consider in the development of commercially viable cultivars with the *pha1pha1pha2pha2* genotype for LP.

Phytate P in soybean meal is largely unavailable to swine, poultry, and other nonruminant animals because they have little or none of the phytase enzyme in their digestive systems (Erdman, 1979). Phytate P binds to nutritionally beneficial metals including zinc, calcium, and magnesium, which reduces their availability to nonruminants (Raboy et al., 1984). Reducing phytate P and increasing inorganic P in soybean meal would increase the amount of P available to nonruminants, decrease the amount of supplemental inorganic P added to their ration, and lower their fecal P (Cromwell et al., 2000; Spencer et al., 2000; Cromwell, 2002).

Conventional soybean seed contains about 4.3 g kg^{-1} phytate P and 0.7 g kg^{-1} inorganic P (Wilcox et al., 2000). A mutant line with reduced phytate P and increased inorganic P was developed by chemical mutagenesis by Wilcox et al. (2000). They crossed the LP mutant line to the cultivar Athow to develop the LP breeding line CX1834-1-6. Oltmans et al. (2004) found that LP in CX1834-1-6 was controlled by recessive alleles at two independent loci that were designated *pha1* and *pha2*. The two alleles exhibit duplicate dominant epistasis, and only individuals homozygous for the two recessive alleles have LP.

The influence of LP on the agronomic and seed traits of soybean lines homozygous for *pha1* and *pha2* is not known. Meis et al. (2003) compared LP soybean lines homozygous for the *mips* allele with NP lines with the *Mips* allele and observed that the *mips* lines had significantly less seedling emergence, particularly when the seed was produced in semitropical locations. The objective of our study was to evaluate the agronomic and seed traits of LP lines with the *pha1pha1pha2pha2* genotype in comparison with NP lines from the same single-cross populations.

MATERIALS AND METHODS

Three single-cross populations were developed by crossing a LP line to three high-yielding NP parents. The LP line CX1834-1-6 with the genotype *pha1pha1pha2pha2* was obtained from J.R. Wilcox, USDA-ARS and Purdue University. It was selected from a population developed by crossing Athow (Wilcox and Abney, 1997) to the mutant line M153-1-4-6-14. M153-1-4-6-14 was obtained by treating the breeding line CX1515-4 with ethyl methanesulfonate (Wilcox et al., 2000).

The three NP parents used in this study were developed by Iowa State University: 'IA1008', 'IA2050', and 'IA2068'. IA1008 was selected from the cross of 'S20-20' × 'Jack'. S20-20 was a cultivar developed by the Northrup King Co., Minneapolis, MN. Jack was developed by the University of Illinois, Urbana, IL (Nickell et al., 1990). IA2050 was selected from the cross of 'S24-92' × A91-501002. S24-92 was developed by Northrup King Co., Minneapolis, MN. A91-501002 was an experimental line developed by Iowa State University. IA2068 was developed from the cross of 'AP1953' × LN94-10470. AP1953 was developed by AgriPro Seeds, Ames, IA. LN94-10470 was developed by the University of Illinois.

The crosses to form the three populations were made in July 2001 at the Agricultural and Agronomy Research Center near Ames, IA. The cross of IA1008 × CX1834-1-6 was designated as Population 1, CX1834-1-6 × IA2050 as Population 2, and IA2068 × CX1834-1-6 as Population 3. The F_1 seeds from each cross were planted during October 2001 at the Iowa State University-University of Puerto Rico soybean breeding nursery at Isabela, PR. The soil type is a Coto clay (very-fine, kaolinitic, isohyperthermic Typic Eutrustox). The F_1 plants of Population 1 were confirmed as hybrid by flower color. IA1008 had white flowers, CX1834-1-6 had purple flowers, and the hybrid plants had purple flowers. For Populations 2 and 3, leaves from the F_1 plants were harvested and analyzed with DNA markers to confirm hybrids. The confirmed F_1 plants of each population were threshed in bulk.

For each population, 350 F_2 seeds and 50 seeds of each parent were planted at Isabela, PR, during February 2002. Each F_2 plant was harvested individually and the seed of each parent was harvested in bulk.

Two sets of 110 entries were planted for each population in May 2002 at the Agricultural and Agronomy Research

S.E. Oltmans, W.R. Fehr, and G.A. Welke, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011; V. Raboy and K.L. Peterson, USDA-ARS, 1691 South 2700 West, Aberdeen, ID 83210. This journal paper of the Iowa Agric. and Home Econ. Exp. Stn., Ames, IA, Project No. 3732 was supported by the Hatch Act, State of Iowa, Iowa Soybean Promotion Board, Raymond F. Baker Center for Plant Breeding, and the United Soybean Board. Received 5 Feb. 2004. Crop Breeding, Genetics & Cytology. *Corresponding author (wfehr@iastate.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: LP, low phytate; NP, normal phytate.

Center near Ames, IA. Each set contained 105 $F_{2:3}$ lines from a population and five checks, which were the two parents used to form the population and three high-yielding NP cultivars or lines of different maturity groups. Each set was grown as a randomized complete-block design with one replication planted at the Agronomy Farm and one replication at the Burkey Farm. The soil type at both locations is a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll). For each plot, 20 seeds were planted in single rows 0.76 m long with a row spacing of 1.02 m between plots and a 1.07 m alley between the ends of plots.

Maturity date was recorded when 95% of the pods in a plot had reached their mature color. Each plot was harvested in bulk with a single-row self-propelled combine (Almaco, Nevada, IA). After harvest, 23 individual seeds from each $F_{2:3}$ line were evaluated for the high inorganic P phenotype associated with the low phytate trait by the colorimetric assay adapted from Chen et al. (1956), Raboy (2000), and D.W. Israel (personal communication, 2001). It was necessary to evaluate 23 individual seeds to have a 95% probability of differentiating the homogeneous NP lines that originated from F_2 plants with the genotypes *Pha1Pha1Pha2Pha2, Pha1Pha1Pha2pha2, Pha1pha1Pha2Pha2, Pha1Pha1pha2pha2*, and *pha1pha1Pha2Pha2* from the heterogeneous lines that originated from the F_2 genotype *Pha1pha1Pha2pha2* (Sedcole, 1977). The test also identified the homogeneous LP lines. After testing, 10 LP and 10 NP $F_{2:4}$ lines were selected from each population. Each LP line was matched with an NP line of similar maturity to minimize the influence of maturity on the other agronomic and seed traits.

The 20 lines selected from each cross were grown as a separate experiment in a randomized complete-block design with two replications at Ames, Carlisle, and Rippey, IA, in May 2003. The Ames location was at the Agronomy Farm. The soil type at Carlisle is a Tama silty clay loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) and at Rippey is a Nicollet loam (fine-loamy, mixed, mesic Aquic Hapludoll). The two-row plots were 3.05 m long with 0.69 m between rows within a plot, 1.02 m between rows of adjacent plots, and a 0.91 m alley between the ends of plots. The seeding rate was 30 seeds m^{-1} of row.

Total P, phytate P, and inorganic P were measured on two replications of the $F_{2:4}$ seed used for planting in 2003. Samples of mature seeds for each replication were dried for 48 h at 60°C, milled to pass through a 20-mm screen, and stored in a desiccator until analysis. Total P was determined following wet-ashing of 150 mg of a ground sample with a colorimetric assay of digest P (Chen et al., 1956). Inorganic P was determined colorimetrically following extraction of 0.5 g of a ground sample in 12.5% (w/v) TCA and 92 m*M* $MgCl_2$. The ferric-precipitation method was used to determine phytate P (Raboy et al., 2000). A 0.5-g sample was extracted in 0.4 *M* HCl:0.7 *M* Na_2SO_4. Phytate P was obtained as a ferric precipitate, wet-ashed, and assayed for P as in the total P analysis. Phytate P was expressed as its P (atomic weight 31) content to facilitate comparisons between seed P fractions. To confirm the accuracy of the ferric-precipitation method, phytate P also was analyzed in each sample comprising the first replicate using an anion-exchange HPLC method for seed phytate P as described in Dorsch et al. (2002). The values for phytate P obtained via the ferric-precipitation method were found to be in good agreement with the values obtained via HPLC. Other P was determined by subtracting phytate P and inorganic P from total P. Other P represented the sum of nonphytate P and noninorganic P compounds including RNA, DNA, protein, lipids, and starches.

Data were collected on all plots for seedling emergence, plant density, yield, maturity, height, lodging, seed size, protein, oil, and fatty ester content. Emergence and plant density were determined by counting the number of plants in a plot at the V2 stage, when the trifoliate at the node above the unifoliate was fully developed (Fehr and Caviness, 1977). Emergence percentage was computed by dividing the number of plants in a plot by the 180 seeds planted in each plot and multiplying the quotient by 100. The number of plants in a plot was converted to plant density in plants per square meter. Maturity was recorded as the number of days after 31 August when 95% of the pods in a plot had reached their mature color. Height was measured in centimeters at plant maturity as the distance from the soil surface to the terminal node. Lodging was recorded at maturity on a scale of 1 (erect) to 5 (prostrate). The plots were harvested with a two-row self-propelled plot combine (Almaco, Nevada, IA), and the weight and moisture content of the seed were recorded. Yield of each plot was adjusted to 130 g kg^{-1} moisture. Seed size for each plot was measured by weighing 400 whole seeds. Protein, oil, and moisture content were measured on a 300-g sample with a whole grain near-infrared reflectance analyzer (Infratec, Hooganas, Sweden). Protein and oil content were adjusted to 130 g kg^{-1} moisture. Fatty ester content was measured on two five-seed samples per plot by gas chromatography as described by Hammond (1991). The mean of the two samples for a plot was used for analysis of the fatty ester data.

The data for each trait were analyzed as a randomized complete-block design by the linear model procedure of the SAS statistical software (release 8.02)(SAS Institute, 2001). Replications and environments were considered random effects. Types and genotypes within types were considered fixed effects. The significance of the main effects and interactions were determined by an *F* test. The environment × main effect interactions were used to test the significance of the main effects for all traits in the combined analysis of variance across environments. Phenotypic correlations among traits were determined using the CORR procedure of SAS statistical software.

RESULTS AND DISCUSSION

The mean total P content of the LP and NP lines was not significantly different in any of the populations (Table 1). The proportion of phytate P, inorganic P, and other P in the two types of lines was significantly different in all of the populations. Averaged across populations, the LP lines had 24.9% phytate P, 37.9% inorganic P, and 37.2% other P while the NP lines had 71.4% phytate P, 3.8% inorganic P, and 24.8% other P.

The reduced phytate P and increased inorganic P for the LP lines was expected on the basis of previous research (Wilcox et al., 2000). Previous research did not report the differences between LP and NP genotypes for other P. The significantly greater other P in LP lines compared with NP lines in all populations indicated that there was an increase in one or more of the other P-containing compounds in the LP seed. Additional research will be needed to confirm that other P is increased in LP seeds compared with NP seeds and to identify the other P-containing compounds that are increased.

The mean seedling emergence percentage and plant density for the LP lines was significantly lower than for the NP lines in the three populations (Table 1). Averaged across populations, the LP lines were 23% units lower in emergence and 7.9 plants m^{-2} lower in plant

Table 1. Mean agronomic and seed traits of 10 low- and 10 normal-phytate soybean lines from each of three populations.

		Population					
		1		2		3	
Trait	Type‡	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range
Total P, mg g^{-1}§	LP	8.23ns†	7.68–8.92**	7.71ns	7.13–8.62*	7.94ns	7.37–8.97*
	NP	8.12	7.49–8.55*	7.78	7.25–8.30ns	7.96	7.54–8.44ns
Phytate P, mg g^{-1}§	LP	2.07**	1.72–2.45ns	1.97**	1.72–2.74ns	1.90**	1.71–2.29ns
	NP	5.74	5.07–6.12*	5.51	5.05–6.18ns	5.78	4.79–6.34**
Inorganic P, mg g^{-1}§	LP	3.20**	2.70–3.53**	2.77**	2.21–3.27**	3.10**	2.68–4.09**
	NP	0.31	0.25–0.65ns	0.30	0.24–0.47ns	0.30	0.26–0.37ns
Other P, mg g^{-1}§#	LP	2.96**	2.62–3.21ns	2.97**	2.58–3.41ns	2.94**	2.65–3.74ns
	NP	2.07	1.54–2.52ns	1.97	1.82–2.46ns	1.88	1.57–2.56ns
Emergence, %¶	LP	41*	16–54**	54*	36–66**	41**	19–54**
	NP	64	52–72**	73	60–79**	68	62–74*
Plant density, plants m^{-2}¶	LP	14.2*	5.4–18.6**	18.6*	12.5–22.9**	14.2**	6.7–18.8**
	NP	22.1	18.0–24.9**	25.3	21.0–27.3**	23.4	21.6–25.7*
Yield, kg ha^{-1}¶	LP	1616*	975–1993**	1711ns	1278–2009**	1778ns	1277–2094**
	NP	1993	1546–2293**	1842	1607–2225**	2076	1892–2415*
Maturity, d¶##	LP	18ns	13–20**	16ns	11–18**	17ns	14–19**
	NP	16	9–20**	14	10–17**	15	12–19**
Lodging, score¶##	LP	2.1ns	1.7–2.6*	1.8ns	1.7–1.9ns	1.9ns	1.8–1.9ns
	NP	2.0	1.6–2.4**	1.8	1.7–2.1ns	2.0	1.8–2.3ns
Height, cm¶	LP	96ns	79–113**	77ns	70–85**	79**	73–90**
	NP	100	82–118**	79	72–83**	85	75–96**
Seed size, mg sd^{-1}¶	LP	143ns	134–164**	131ns	116–144**	131ns	122–145**
	NP	140	124–153**	128	110–142**	127	114–145**
Protein, g kg^{-1}¶	LP	375ns	354–400**	374*	363–396**	362ns	349–380**
	NP	372	362–383*	389	376–400**	369	350–386**
Oil, g kg^{-1}¶	LP	171**	151–184**	174*	150–185**	181ns	171–187**
	NP	178	168–188**	168	153–180**	181	169–190**
Palmitate, g kg^{-1}¶	LP	119*	114–122*	116ns	99–122**	116**	111–120**
	NP	114	107–123**	115	110–122**	109	98–116**
Stearate, g kg^{-1}¶	LP	54**	46–63**	51ns	47–54**	52ns	48–59**
	NP	48	45–51**	48	45–50ns	50	46–57**
Oleate, g kg^{-1}¶	LP	231ns	205–242**	244*	232–267**	242ns	227–269**
	NP	233	223–263**	237	226–247**	242	231–268**
Linoleate, g kg^{-1}¶	LP	524**	513–548**	517ns	505–528*	520*	499–538**
	NP	533	515–545**	522	512–530*	528	505–539**
Linolenate, g kg^{-1}¶	LP	72ns	67–83**	74*	54–83**	69ns	63–75**
	NP	73	68–78**	79	76–83*	71	67–74*

* Differences between the means of the two types or the means of the lines within each type were significant at the 0.05 probability level.
** Differences between the means of the two types or the means of the lines within each type were significant at the 0.01 probability level.
† ns = Differences between the means of the two types or the means of the lines within each type were not significant at the 0.05 probability level.
‡ LP = low-phytate lines, NP = normal-phytate lines.
§ Mean of two replications of analysis for seed planted at three Iowa environments in 2003.
¶ Mean of two replications at each of three Iowa environments in 2003.
# Other P = total P − (phytate P + inorganic P).
‡‡ Days after 31 August.
Lodging score = 1.0, plants erect, to 5.0, plants prostrate.

density than the NP lines. Hulke et al. (2004) compared the emergence of LP lines with the *pha1pha1pha2pha2* genotype and NP lines. All of their lines had reduced palmitate, instead of the normal palmitate of lines in our study. Their LP lines had 22.3% units less emergence than the NP lines. Meis et al. (2003) also observed a reduction in seedling emergence for LP lines with the *mips* allele. The percentage reduction in emergence of their *mips* lines was influenced by the environment in which the seed was produced. The *mips* lines grown from seed obtained from temperate seed sources had a mean field emergence of 63%, while the same lines grown from seed obtained from subtropical seed sources had a mean field emergence of 8%. *Mips* lines from temperate seed sources had a mean field emergence of 77% compared with a mean field emergence of 83% from subtropical seed sources.

There was a significant difference among environments for seedling emergence of the LP and NP lines in the three populations (Table 2). The LP lines had lower emergence than the NP lines in all of the environments, but the magnitude of the difference between the two types varied across environments, which resulted in a highly significant ($P < 0.01$) environment × type interaction. The inconsistent emergence percentage among environments would make it difficult to predict the seeding rate to use for LP lines in commercial plantings to achieve an acceptable plant density. It may be necessary to develop LP cultivars with normal seedling emergence before LP soybeans can be produced commercially over a range of environments.

The feasibility of developing LP cultivars with normal emergence was evaluated by examining the variation among lines within the LP type (Tables 1, 2, and 3). There were significant differences in emergence among LP lines at each environment and combined across environments for the three populations. The interaction of environment × lines within LP type was significant ($P < 0.05$) for Population 1, but was not significant for the other two populations. It should be possible to select for improved seedling emergence among LP lines.

If increased emergence was due to an increase in

Table 2. Mean seedling emergence of 10 low- and 10 normal-phytate soybean lines from each of three populations at three Iowa environments in 2003.

		Environment					
		Ames		Carlisle		Rippey	
Population	Type‡	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range
		%					
1	LP	24**	7–37**	47**	19–64**	52**	21–70**
	NP	51	41–65**	67	53–73ns†	73	61–83**
2	LP	36**	17–53**	60**	46–80**	65**	46–82**
	NP	63	48–71*	75	65–82*	81	66–90**
3	LP	26**	9–38**	47**	27–61**	51**	22–69**
	NP	57	51–66ns	70	61–79ns	75	68–80ns

* Differences between the means of the two types or the means of the lines within each type were significant at the 0.05 probability level.
** Differences between the means of the two types or the means of the lines within each type were significant at the 0.01 probability level.
† ns = Differences between the means of the two types or the means of the lines within each type were not significant at the 0.05 probability level.
‡ LP = low-phytate lines, NP = normal-phytate lines.

Table 3. Mean agronomic and seed traits of 10 low-phytate lines in each of three populations.

Entry	Emergence†	Plant density†	Total P‡	Phytate P‡	Inorganic P‡	Other P‡§	Yield†
	%	plants m^{-2}	mg g^{-1}				kg ha^{-1}
Population 1							
747009	16	5.4	8.92	2.24	3.53	3.15	975
747011	33	11.6	8.00	2.33	2.79	2.88	1649
747015	35	12.1	8.67	2.24	3.52	2.91	1526
747017	38	13.2	8.06	1.72	3.22	3.12	1627
747003	40	13.7	8.52	1.98	3.50	3.04	1539
747013	41	14.1	8.01	2.45	2.70	2.86	1677
747001	50	17.2	8.15	1.86	3.08	3.21	1793
747005	51	17.8	7.68	1.84	3.11	2.73	1642
747007	53	18.2	7.91	2.12	3.17	2.62	1993
747019	54	18.6	8.34	1.88	3.36	3.10	1736
LSD 0.05	6	2.1	0.44	0.37	0.45	0.42	225
Population 2							
747029	36	12.5	8.61	1.93	3.27	3.41	1278
747037	48	16.7	8.23	1.80	3.10	3.33	1628
747033	51	17.7	7.12	1.91	2.42	2.79	1754
747027	52	18.1	7.85	1.71	2.90	3.24	1462
747025	53	18.4	7.32	1.95	2.79	2.58	1663
747031	55	19.0	7.61	2.74	2.21	2.66	1809
747021	57	19.7	7.35	1.88	2.70	2.77	1831
747023	57	19.9	7.53	2.00	2.43	3.10	2009
747035	60	20.9	7.61	1.80	2.78	3.03	1783
747039	66	22.9	7.89	2.02	3.05	2.82	1897
LSD 0.05	7	2.4	0.94	0.36	0.34	0.85	224
Population 3							
747045	19	6.7	8.87	1.91	4.09	2.87	1277
747041	30	10.5	8.97	2.29	2.94	3.74	1570
747043	33	11.6	7.87	1.93	2.96	2.98	1673
747055	37	12.8	7.82	1.96	3.06	2.80	1770
747053	39	13.5	7.91	1.78	3.23	2.90	1779
747051	45	15.7	7.78	1.81	3.08	2.89	2094
747047	49	17.0	7.37	1.73	2.99	2.65	1809
747059	51	17.7	7.59	1.71	3.03	2.85	1801
747049	52	18.1	7.61	1.96	2.94	2.71	2067
747057	54	18.8	7.64	1.96	2.68	3.00	1936
LSD 0.05	6	2.0	1.31	0.37	0.44	1.20	231

† Mean of two replications at each of three Iowa environments in 2003.
‡ Mean of two replications of analysis for seed planted at the three Iowa environments in 2003.
§ Other P = total P − (phytate P + inorganic P).

phytate P, it may be difficult to develop cultivars with desirable levels of the two traits. The phenotypic correlation coefficients between emergence percentage and phytate P were positive and significant when all of the lines were included, but were not significant when the LP and NP lines were evaluated independently (Table 4). The correlations between emergence and phytate P for LP lines were not consistent among populations, with negative coefficients in Populations 1 and 3 and a positive coefficient in Population 2. The phytate P of the LP line with the greatest emergence was less than that of the line with the lowest emergence in Population 1, but greater in Populations 2 and 3 (Table 3). The results indicated that selection for increased seedling emergence among LP lines would not consistently result in an increase in phytate P.

Selection for increased seedling emergence among LP lines was associated with a decrease in total P, inorganic P, and other P (Table 3). The phenotypic correlation coefficients between emergence and total P for LP lines were negative for all of the populations and significant for Populations 1 and 3 (Table 4). The correlation coefficients between emergence and inorganic P for LP lines were negative for all the populations, but

Table 4. Phenotypic correlation coefficients between traits for low-phytate, normal-phytate, and all soybean lines from three populations.

Population	Trait	Type†	Yield	Phytate P	Inorganic P	Other P‡	Total P
1	Emergence % and density, plants m^{-2}	LP	0.88**	−0.48	−0.25	−0.34	−0.67*
		NP	0.29	0.45	−0.25	−0.13	0.17
		All	0.83**	0.78**	−0.81**	−0.75**	−0.36
	Yield, kg ha^{-1}	LP		−0.23	−0.47	−0.45	−0.74*
		NP		0.41	−0.15	0.33	0.59
		All		0.64**	−0.68**	−0.56*	−0.23
	Phytate P, mg g^{-1}	LP			−0.32	−0.33	0.23
		NP			−0.82**	−0.27	0.30
		All			−0.99**	−0.88**	−0.12
	Inorganic P, mg g^{-1}	LP				0.35	0.74*
		NP				0.35	−0.02
		All				0.88**	0.23
	Other P, mg g^{-1}‡	LP					0.56
		NP					0.82**
		All					0.48*
2	Emergence % and density, plants m^{-2}	LP	0.83**	0.11	−0.35	−0.55	−0.55
		NP	0.64*	−0.34	−0.66*	0.31	−0.31
		All	0.65**	0.81**	−0.85**	−0.81**	−0.18
	Yield, kg ha^{-1}	LP		0.30	−0.63	−0.57	−0.64*
		NP		−0.20	−0.27	0.05	−0.24
		All		0.30	−0.38	−0.40	−0.41
	Phytate P, mg g^{-1}	LP			−0.59	−0.48	−0.12
		NP			−0.03	−0.39	0.78**
		All			−0.98**	−0.92**	0.14
	Inorganic P, mg g^{-1}	LP				0.61	0.76*
		NP				0.01	0.22
		All				0.93**	0.02
	Other P, mg g^{-1}‡	LP					0.79**
		NP					0.23
		All					0.17
3	Emergence % and density, plants m^{-2}	LP	0.87**	−0.40	−0.73*	−0.39	−0.85**
		NP	0.46	−0.08	−0.65*	0.10	−0.10
		All	0.85**	0.83**	−0.91**	−0.82**	−0.35
	Yield, kg ha^{-1}	LP		−0.28	−0.71*	−0.34	−0.77**
		NP		−0.57	0.15	0.84**	−0.13
		All		0.56**	−0.70**	−0.53*	−0.46*
	Phytate P, mg g^{-1}	LP			−0.14	0.82**	0.67*
		NP			−0.19	−0.78**	0.82**
		All			−0.97**	−0.90**	0.12
	Inorganic P, mg g^{-1}	LP				−0.15	0.56
		NP				0.37	0.12
		All				0.87**	0.07
	Other P, mg g^{-1}‡	LP					0.71*
		NP					−0.30
		All					0.15

* Coefficients were significant at the 0.05 probability level.
** Coefficients were significant at the 0.01 probability level.
† LP = low-phytate lines, NP = normal-phytate lines, All = both LP and NP lines.
‡ Other P = total P − (phytate P + inorganic P).

significant only for Population 3. The correlation coefficients between emergence and other P for LP lines were negative for all of the populations, but were not significant. The LP line with the greatest emergence percentage had lower total P and inorganic P than the line with the lowest emergence for all of the populations and lower other P in Populations 1 and 2, although the differences between the two lines were not always significant. These results indicated that selection for increased emergence among LP lines may result in a decrease in total P, inorganic P, and other P. The physiological basis of the relationship of seedling emergence with total P, inorganic P, and other P in LP lines is not known and would be appropriate to investigate in future research.

The LP lines had significantly lower mean seed yields than the NP lines in all the populations, although the difference between the two types was only significant in Population 1 (Table 1). The differences in yield between the two types were due, at least in part, to their differences in emergence percentage and plant density. The phenotypic correlations of emergence and plant density with yield were highly significant when all lines were included in the analysis, and when the LP lines were considered independently (Table 4). The difference in plant density between the two types of lines made it difficult to assess the influence of the LP on yield. There were LP lines that yielded more than some of the NP lines, which suggested that the LP trait per se might not be associated with a yield reduction (Table 1). On the other hand, the highest yielding line in each population was a NP type. To effectively assess the relationship between the LP trait and yield, the reduction in emergence of LP lines will have to be overcome by additional breeding or the plots of the LP and NP lines will have to be overplanted and thinned to a common plant density.

The lack of significant differences in the mean maturity of the LP and NP lines was expected because each LP line selected for the study was matched with a NP line of similar maturity to minimize the influence of the trait on the other characteristics that were evaluated (Table 1). There was no significant difference between

the two types of lines for lodging score, and the mean plant height was similar between the two types with a maximum difference of 6 cm in Population 3. It should be possible to develop LP cultivars with maturity, lodging, and plant height comparable to NP cultivars.

The mean seed size of LP and NP lines was not significantly different in any of the populations (Table 1). There were no consistent differences between the LP and NP lines in the three populations for protein, oil, and fatty ester content. The ranges among lines for the two types were similar for all the seed traits, which indicated that it should be possible to develop LP cultivars that are similar to NP cultivars for the seed traits that were evaluated in the study.

The primary challenge for development and commercialization of LP cultivars with the *pha1pha1pha2pha2* genotype will be the reduction in seedling emergence. Additional research will be needed to determine if the reduction in emergence can be overcome, if the LP trait has a influence on seed yield independent of the reduction in emergence, and if the influence of seed source on emergence reported by Meis et al. (2003) for LP lines with the *mips* allele also applies to lines with *pha1* and *pha2* alleles.

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